Hi Dan,

I have also got mCherry positive lines, by PCR.

But they are not so easy to  visualize under microscope.

Will have a look at them again after passing them through spore stages.

But in general, I have an issue that tansformants are not soprulating that good., when I put them from the cell culture plate to Petriplate.

It has started, but is taking time. For some reason they are all showing more fluffy growth. It might have to do something with the timing: temperature/ light

As it is a general trend.  I think I have to wait a bit more for the second round of subculture.

         I would like to get the SNP data (1-2 kb, up flanking and down flanking) region of genes, I would like to make Knockouts of.

             If I can have access to the vcf file that might be enough, I hope I will be able to extract SNPs from these files.

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| --- | --- | --- |
| Gene ID | Annotation | Priority/comments |
| Bcin13g00710.1 | ABC-transporter extracellular N-terminal (Control) | Get in from Henk Jan |
| Bcin06g05230.1 | GAL4-like Zn2Cys6 binuclear cluster DNA-binding domain | 1 |
| Bcin12g05690.1 | Thioredoxin like superfamily | 3 |
| Bcin06g00026.1 | MFS\_transporter | Try in Xenopus first |
| Bcin12g06180.1 | Nitrilases, cyanide hydratase (CH)s, | 2 |
| Bcin05g04960.1 | Nitrilases, cyanide hydratase (CH)s | 2 |
| Bcin01g01260.1 | Type 1 glutamine amidotransferase (GATase1) | 2 |
| Bcin09g01110.1 | Glycosyl hydrolase family 7 | 3 |
| Bcin15g05080.1 | Glycosyl hydrolase family 1 | 3 |
| Bcin08g03460.1 | short chain dehydrogenase; | 3 |
| Bcin03g04480.1 | SDR superfamily | 3 |
| Bcin11g01310.1 | alpha/beta hydrolases | 3 |

         Regarding the lusiferase gene:

              As I said in my mail before, It should be pretty easy to transform Botrytis with luciferase gene. I could synthesize a codon-optimized luciferase gene and transform it into Botrytis., under a constitutive promoter.

It might be cool to generate to  bioluminescent Botrytis (without adding any substrate) . However cloning 6-7 genes and engineering a whole pathway could be challenging., and might be a project on its own, if it needs codon-optimization and other optimizations. If I can get the constructs, may be it is worth a try. As positives can be screened easily by eyes.

Just out of curiosity, do you know why they need bioluminescent Botrytis? What are they going to use it for?